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FATE OF MICRO-ORGANISMS INTRODUCED INTO ISOLATED LOOPS OF THE INTESTINE *

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It is currently assumed that pathogenic organisms are killed or at least inhibited in their multiplication in the intestinal tract, mainly by the antagonistic action of the normal intestinal flora. This assumption is the basis for numerous suggested methods of bacteriotherapy which all have as their object the implantation of nonpathogenic antagonistic forms in the intestinal canal. We have attempted to test the validity of this fundamental assumption by studying the fate of micro-organisms introduced into isolated loops of the intestine.

Medium sized dogs were used. Under morphine-ether anesthesia, the abdomen was opened, and a ligature placed around the duodenum immediately below the pylorus. The duodenum was then flushed out with warm, physiologic salt solution introduced through a hypodermic needle inserted immediately below the ligature. During the washing-out process the duodenum was repeatedly milked downward to force out the solution. After the washing, a 20-30 cm. loop of the emptied duodenum was isolated between ligatures. This loop always included the entrances of the bile and pancreatic ducts. By the same method a 20-30 cm. jejunal loop was isolated.

Of the bacterial suspension, 5 cc were now injected into each loop by means of a hypodermic needle. The suspension usually contained from 5,000,000 to 150,000,000 organisms per c.c. The contents of each loop were now thoroughly mixed by gentle massage, and a 3 cc sample of the mixed contents was removed for a study of bacterial antagonisms. This left approximately 2 cc of the original bacterial suspension in each loop.

The abdomen was then closed and the dog allowed to recover from the anesthetic. After a period, usually varying from 2 to 5 hours, occasionally as long as 12 hours, the animal was killed and the contents of each loop removed. The total number of residual specific micro-organisms in each loop was determined by the ordinary dilution method of milk and water analysis. Aerobic plates only were studied.

Data thus obtained from the use of 26 dogs are recorded in table 1.

Table 1 shows that *S. lutea* completely disappears from both loops of the intestine within 2 hours, and *B. violaceus* within 5 hours. *S. aureus* is greatly reduced in number in one loop within 3 hours and completely disappears from both loops within 12 hours. Even the spores of *B. subtilis* completely disappear from both loops within

that period of time. Red yeast disappears from one loop within 12 hours. *B. typhosus* is greatly reduced in number within 5 hours, but whether or not there is a complete disappearance of this micro-organism cannot be ascertained with the technic used.

TABLE 1
SPECIFIC ORGANISMS RECOVERED FROM ISOLATED LOOPS OF THE INTESTINE

The total number of bacteria injected into each loop usually varied from 10,000,000 to 300,000,000. In order to simplify the table, the number injected in each case has been recorded as 100%.

Micro-organism	Time in Hours	Percentage Recovered		
		Duodenal Loop, Percentage	Jejunal Loop, Percentage	Average Percentage
<i>S. lutea</i>	1½	0	50	25
	2	0	0	0
	4	0	0	0
	7	0	0	0
<i>B. violaceus</i>	2	180	30	105
	3	110	10	60
	5	0	0	0
	7	0	0	0
<i>S. aureus</i>	3	130	30	80
	5	125	10	65
	7	15	5	10
	12	0	0	0
<i>B. subtilis</i> (contained spores)...	2	100*	100*	100
	12	0	0	0
<i>B. typhosus</i>	5	1†	1†	1
Red yeast.....	3	90	120	105
	5	185	130	155
	12	0	125	60
Streptococcus.....	3	50‡	105‡	80
	5	110‡	120‡	115
<i>B. anthracis</i> (contained spores)	3	100*	100*	100
	5	100*	100*	100
	12	70	140	105

* Approximate count.

† Total count of intestinal aerobes, mainly *B. coli*.

‡ Total count of hemolytic colonies.

The experiments with streptococci are inconclusive, due to the probable presence of other hemolytic organisms. *B. anthracis* spores are apparently not killed.

At the time of the necropsy, the duodenal loop always contained bile-stained fluid, the total fluid contents usually varying from 5 to 10 c c, occasionally being as great as 60 c c. The jejunal loop usually contained but from 1 to 2 c c of a slightly viscid fluid. This loop was usually washed out with salt solution to make the count.

Table 1 shows a more rapid disappearance of the micro-organisms from the jejunal loop in the majority of cases.

In order to test the rôle of bacterial antagonism in this disappearance, samples of the intestinal contents withdrawn from each loop immediately after the injection of the micro-organisms were incubated,

under both aerobic and anaerobic conditions. Counts of these incubated samples were made at the time of the necropsy. Data thus obtained are recorded in table 2.

TABLE 2
TEST OF BACTERIAL ANTAGONISMS

The original number of micro-organisms injected in each case is recorded as 100%.

Micro-organism	Time in Hours	Specific Micro-organisms in Incubated Controls		Other Aerobic Micro-organisms Present
		Aerobic, Percentage	Anaerobic, Percentage	
<i>S. lutea</i>	1½	120	150	1.5
	2	80	...	0
	4
	7	0	0	1000
<i>B. violaceus</i>	2	160	50	0.5
	3	105	70	5
	5	60	70	70
<i>S. aureus</i>	3	50	80	10
	5	85	85	...
	7	90	80	...
	12	1200	1800	1000
<i>B. subtilis</i> (contained spores)....	2	100*	100*	...
	12	75	65	50
<i>B. typhosus</i>	5
Red yeast.....	3	80	80	1
	5	125	100	1
	12	125	100	500
<i>Streptococcus</i>	3	105†
	5	160†	180	...
<i>B. anthracis</i> (contained spores)..	3	100*	100*	...
	5	100*	100*	...
	12	110	120	...

* Approximate counts.

† Total count of hemolytic colonies.

Table 2 shows little, if any, bacterial destruction in the incubated controls, except in the case of *S. lutea*. In certain of the longer incubations, e. g., *S. aureus*, there is even a considerable multiplication of the specific micro-organisms in spite of the presence of numerous presumable antagonistic forms. Multiplication is apparently inhibited with other micro-organisms, e. g., *B. violaceus* and *B. subtilis*; but whether or not this is due solely to bacterial antagonisms cannot be determined by the technic used.

CONCLUSIONS AND SUMMARY

Many specific micro-organisms injected into isolated loops of the intestine of dogs are destroyed in periods ranging from two to seven hours.

This destruction is not due to the antagonistic action of the normal intestinal flora.

There is evidently a distinct antibacterial mechanism in the intestine, the nature of which is at present unknown.